Corporation obtaining approval, the name of its representative, and the address of its main office

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Approved Type 1 Use Regulation

Name of the Type of Living Modified Organism	Oilseed rape tolerant to glyphosate herbicide (*cp4 epsps*, *gox*, *Brassica napus* L.) (RT200, OECD UI : MON-89249-2)
Content of the Type 1 Use of Living Modified Organism	Provision as food, provision as feed, cultivation, processing, storage, transportation, disposal and acts incidental to them
Method of the Type 1 Use of Living Modified Organism	—

Outline of the Biological Diversity Risk Assessment Report

I. Information concerning preparation of living modified organisms

(1) Information concerning donor nucleic acid

i) Composition and origins of component elements

Composition of the donor nucleic acid that was used for the development of glyphosate tolerant oilseed rape (*cp4 epsps*, *gox*, *Brassica napus* L.) (RT200, OECD UI : MON-89249-2) (hereinafter referred to as “this recombinant oilseed rape”) and the origins of component elements are shown in Table 1. The *cp4 epsps* gene and the *gox* gene, which were used for the development of this recombinant oilseed rape, have their nucleotide sequences modified to enhance the expression in plants. Regarding the amino acid sequences, CP4 EPSPS protein, which expresses in this recombinant oilseed rape, has the serine, the 2nd amino acid in the N-terminal sequence, replaced by leucine, when compared to the wild-type CP4 EPSPS protein. On the other hand, GOX protein, which expresses in this recombinant oilseed rape, contains the wild-type amino acid sequences. Therefore, among the genes introduced into this recombinant oilseed rape and the proteins expressed by these genes, those subjected to the modification are referred to as "modified *cp4 epsps* gene," and "modified CP4 EPSPS protein."

The only difference of this recombinant oilseed rape from the oilseed rape tolerant to glyphosate herbicide which has been already approved in Japan (*cp4 epsps*, *gox*, *Brassica napus* L.) (RT73, OECD UI : MON-ØØØ73-7) (hereinafter referred to as "RT73") is that, for the gene of glyphosate degradation enzyme in the transferred vector, this recombinant oilseed rape contains the same amino acid sequences as the wild type while the gene in RT73 is a variant of *gox* gene (=*gox v247* gene) which has the amino acids obtained from mutagenesis modified at three (3) positions.

The nucleotide sequences of all the component elements of this recombinant oilseed rape are shown in Annex 2.

ii) Function of component elements

Functions of component elements of donor nucleic acid that was used for the development of this recombinant oilseed rape are shown in Table 1.

[Modified *cp4 epsps* gene]

(a) Glyphosate herbicide is the active ingredient of Roundup, a nonselective herbicide, and inhibits the activity of 5-enol-pyruvylshikimate-3-phosphate synthase (EPSPS) (E.C.2.5.1.19), one of the enzymes in the shikimate pathway for aromatic amino acid biosynthesis, by specifically binding to the enzyme (Reference 38; Reference 39). As a result, plants treated with glyphosate cannot synthesize aromatic amino acid essential for protein synthesis due to the inhibition of EPSPS, and die. The modified *cp4 epsps* gene, a target gene of

this recombinant oilseed rape, expresses the modified CP4 EPSPS protein which has high tolerance to the herbicide glyphosate. The activity of the modified CP4 EPSPS protein to be produced by the modified *cp4 epsps* gene is not inhibited even under the presence of glyphosate, thus the recombinant plants that express this protein have normal functions of shikimate pathway and can grow.

EPSPS is one of the enzymes that catalyze the shikimate pathway for aromatic amino acid biosynthesis, which is unique to plants or microorganisms, and EPSPS is located in chloroplasts or plastids in plants (Reference 40). The shikimate pathway is an important metabolic pathway that is considered to be involved in one fifth of carbon fixation by plants (Reference 41; Reference 39). This pathway is regulated by 3-deoxy-D-arabino-heptulonate-7-phosphate (DAHP) synthase, which is involved in the first step of the pathway, but it has been clarified to be extremely unlikely that the stages from DAHP to the synthesis of chorismic acid through the production of 5-enol-pyruvylshikimate-3-phosphate (EPSP) which is catalyzed by the EPSPS are inhibited or suppressed by metabolic intermediates or end products of this pathway (Reference 42; Reference 43). This suggests that EPSPS is not a rate-determining enzyme in this pathway, and as such it is not considered that enhanced EPSPS activity will increase the concentration of aromatic amino acids, the end products of this pathway. In practice, it is reported that plant cells that produce 40 times as much EPSPS as compared to normal do not synthesize excessive aromatic amino acids (Reference 44). In addition, Monsanto Co. examined amino acid content in the seeds of the recombinant crops in the process of food/feed safety assessment of crop plants (soybean, cotton and maize) that have been commercialized with the feature of tolerance to the Roundup herbicide, and confirmed that there is no difference in the aromatic amino acid content between the original non-recombinant plants and recombinant plants. These facts support the theory that EPSPS is not the rate-determining enzyme in this pathway. Besides, EPSPS is the enzyme that catalyzes a reversible reaction to produce EPSP and inorganic phosphate (Pi) from phosphoenolpyruvate (PEP) and shikimate-3-phosphate (S3P) (Reference 45), and it is known to specifically react with these substrates (Reference 46). The only substance that is known to react with EPSPS other than these is shikimate, an analogue of S3P, but the reactivity with shikimate is only one two millionth of the reactivity with S3P, and it is unlikely that shikimate exerts any significant influence on the metabolic system of plant body even if it reacts.

Compared with the wild-type CP4 EPSPS protein, the modified CP4 EPSPS protein expressed in this recombinant oilseed rape contains the leucine, the 2nd amino acid in the N-terminal sequence, replaced by serine. This modification is conducted in order to produce restriction enzyme of *Nde*I in the N-terminal side.

In addition, the modified *cp4 epsps* gene contains *AEPSPS/CTP2* gene, a *chloroplast transit peptide* (*CTP*) gene derived from *Arabidopsis thaliana*, bonded at the 5'-terminal. This gene serves to facilitate the transfer of translated proteins to chloroplast and enhance the expression in plant body (Reference 47).

(b) In order to investigate whether the modified CP4 EPSPS protein shares functionally important amino acid sequence with known allergens, the modified CP4 EPSPS protein was compared with known allergens in the database (GenBank, EMBL, PIR, NRL3D, SwissProt). As a result, the modified CP4 EPSPS protein did not share structurally related homologous sequences with any of the known allergens examined.

[*gox* gene]

(a) Glyphosate is degraded and inactivated by microorganisms in soil. This is attained based on the process that the enzyme for degradation of glyphosate (Glyphosate Oxidoreductase; GOX) in microorganisms degrades the herbicide glyphosate into aminomethylphosphonic acid (AMPA) and glyoxylate, both of which offer no herbicidal activity and are commonly found in a number of gram-negative and gram-positive bacteria degrading the herbicide glyphosate (Reference 48; Reference 49). Then, among the organisms estimated to offer the capability of degrading the glyphosate into AMPA and glyoxylate, *Ochrobactrum anthropi* (designation based on former classification: *Achromobactor* sp.) LBAA strain was selected which showed the highest capability of glyphosate degradation, and the *gox* gene was isolated (Reference 50; Reference 51). The *Ochrobactrum anthropi* LBAA strain is reportedly one of the organisms existing most in number in the rhizosphere of plants (Reference 52), and it is known that *Ochrobactrum anthropi* LBAA strain could utilize glyphosate as carbon and/or phosphorus sources (Reference 51). In addition, the GOX protein is an enzyme which catalyzes the degradation of glyphosate into AMPA and glyoxylate, though it has high substrate specificity and then it does not affect the metabolic system in plants (Reference 53).

In addition, the *gox* gene contains *Arab-SSU1A-transit* gene bonded at the 5'-terminal, which is a *CTP* gene derived from a small subunit of *A. thaliana* ribulose bisphosphate carboxylase. This gene serves to facilitate the transfer of translated proteins to chloroplast and enhance the expression in plant body (Reference 47).

(b) In order to investigate whether the GOX protein shares functionally important amino acid sequences with known allergens, the GOX protein was compared with allergens in the database (GenBank, EMBL, PIR, NRL3D, SwissProt). As a result, the GOX protein did not share structurally related homologous sequences with any of the known allergens examined.

Table 1 Component elements of plasmid PV-BNGT03[1]

Component elements	Origin and function
gox gene expression cassette	
FMV promoter	35S promoter of *Figwort mosaic virus* (Reference 54; Reference 55; Reference 56). Involved in the constant expression in all organs of the target gene. It is known that among virus of the genus *Caulimovirus* to which FMV belongs, cauliflower mosaic virus (CaMV) takes the plant of the genus *Brassica* to which oilseed rape belongs as the recipient organism. However, the homology of 35S promoters of FMV and CaMV is low as 10% or less. Therefore, it was considered that the possibility to come in a new virus due to the recombination is extremely low (Annex 6).
Arab-SSU1A-Transit	N-terminal chloroplast transit peptide sequence of small subunit 1A of ribulose-1,5-bisphosphate carboxylase of *Arabidopsis* (Reference 57). Transfers target proteins to chloroplast.
gox	A variant of glyphosate degradation enzyme (glyphosate oxidoreductase; *gox*) gene derived from *Ochrobactrum anthropi* LBAA strain (Reference 51; Reference 58). It has the nucleotide sequences modified to enhance the expression in plant body. It degrades glyphosate into aminomethylphosphonic acid (AMPA) and glyoxylate.
E9 3'	3’ untranslated region of *rbcS E9* gene of pea. Terminates polyadenylation of *gox* gene (Reference 59; Reference 60).
Modified *cp4 epsps* gene expression cassette	
FMV promoter	35S promoter of *Figwort mosaic virus* (Reference 54; Reference 55; Reference 56). Involved in the constant expression in all organs of the target gene. It is known that among virus of the genus *Caulimovirus* to which FMV belongs, *cauliflower mosaic virus* (CaMV) takes the plant of the genus *Brassica* to which oilseed rape belongs as the recipient organism. However, the homology of 35S promoters of FMV and CaMV is low as 10% or less. Therefore, it was considered that the possibility to come in a new virus due to the recombination is extremely low (Annex 6). It also contains 67 bases of the non-transcribed region of the *Arabidopsis thaliana epsps* gene located downstream of the FMV promoter.
AEPSPS/CTP2	N-terminal chloroplast transit peptide sequence of *epsps* gene of *Arabidopsis* (Reference 54; Reference 55; Reference 61). Transfers target proteins to chloroplast.
Modified *cp4 epsps*	5-enol-pyrovylshikimate-3-phosphate (*epsps*) gene derived from *Agrobacterium* sp. strain CP4 (Reference 53). It has the nucleotide sequences modified to enhance the expression in plant body. Expresses modified CP4 EPSPS protein which shows high tolerance to glyphosate herbicide.
E9 3'	3’ untranslated region of *rbcS E9* gene of pea. Terminates polyadenylation of modified *cp4 epsps* gene (Reference 59; Reference 60).

[1] All the rights pertinent to the information in the table above and the responsibility for the contents rest upon Monsanto Japan Limited.

Table 1 Component elements of plasmid PV-BNGT03 (continued) [2]

Component elements	Origin and function
Other component elements	
Right Border (RB)	It is the restriction fragment from pTiT37 plasmid. Initiates the T-DNA transferred from *Agrobacterium tumefaciens* to plant genome (Reference 62).
Left Border (LB)	It is the restriction fragment from Octopine Ti plasmid pTiA6, containing the left border sequence (25 bp) of T-DNA(Reference 63).
ori-V	The replication origin segment in *Agrobacterium* derived from the broad-host range plasmid RK2 (Reference 64).
ori-322	The replication origin of PV-BNGT03 in *E. coli* derived from pBR322 (Reference 65).
aadA	Bacterial promoter, coding sequence, and terminator for an aminoglycoside-modifying enzyme, 3'(9)-O-nucleotidyltransferase from the transposon Tn7. It confers spectinomycin/streptomycin tolerance to bacterial cells (Reference 66). (GenBank accession X03043)
Rop	Coding sequence for repressor of primer protein for maintenance of plasmid copy number in *E. coli.*

2 All the rights pertinent to the information in the table above and the responsibility for the contents rest upon Monsanto Japan Limited.

(2) Information concerning vector

i) Name and origin

The synthetic plasmid vector PV-BNGT03 to be used for developing this recombinant oilseed rape is the plasmid vector which was composed of the plasmid pBR322 derived from *Escherichia coli* and others.

ii) Properties

The total number of base pairs of this vector is 11,479bp, and it possesses modified *cp4 epsps* gene expression cassette and *gox* gene expression cassette (Figure 1). The infectivity of this vector has not been reported.

(3) Method of preparing living modified organisms

i) Structure of the entire nucleic acid transferred in the recipient organism

The T-DNA region of the synthetic plasmid vector PV-BNGT03 to be used for developing this recombinant oilseed rape is composed of *gox* gene expression cassette ([P-FMV]-[Arab-SSU1A-transit]-[*gox*]-[E9 3']) and modified *cp4 epsps* gene expression cassette ([P-FMV]-[AEPSPS/CTP2]-[modified *cp4 epsps*]-[E9 3']), which are both controlled by the FMV promoter. The structure of the nucleic acid and the origin of component elements are shown in Table 1.

ii) Method of transferring nucleic acid transferred in the recipient organism

The T-DNA region of the plasmid vector PV-BNGT03 was introduced to the non-recombinant oilseed rape cultivar, Westar, by the *Agrobacterium* method.

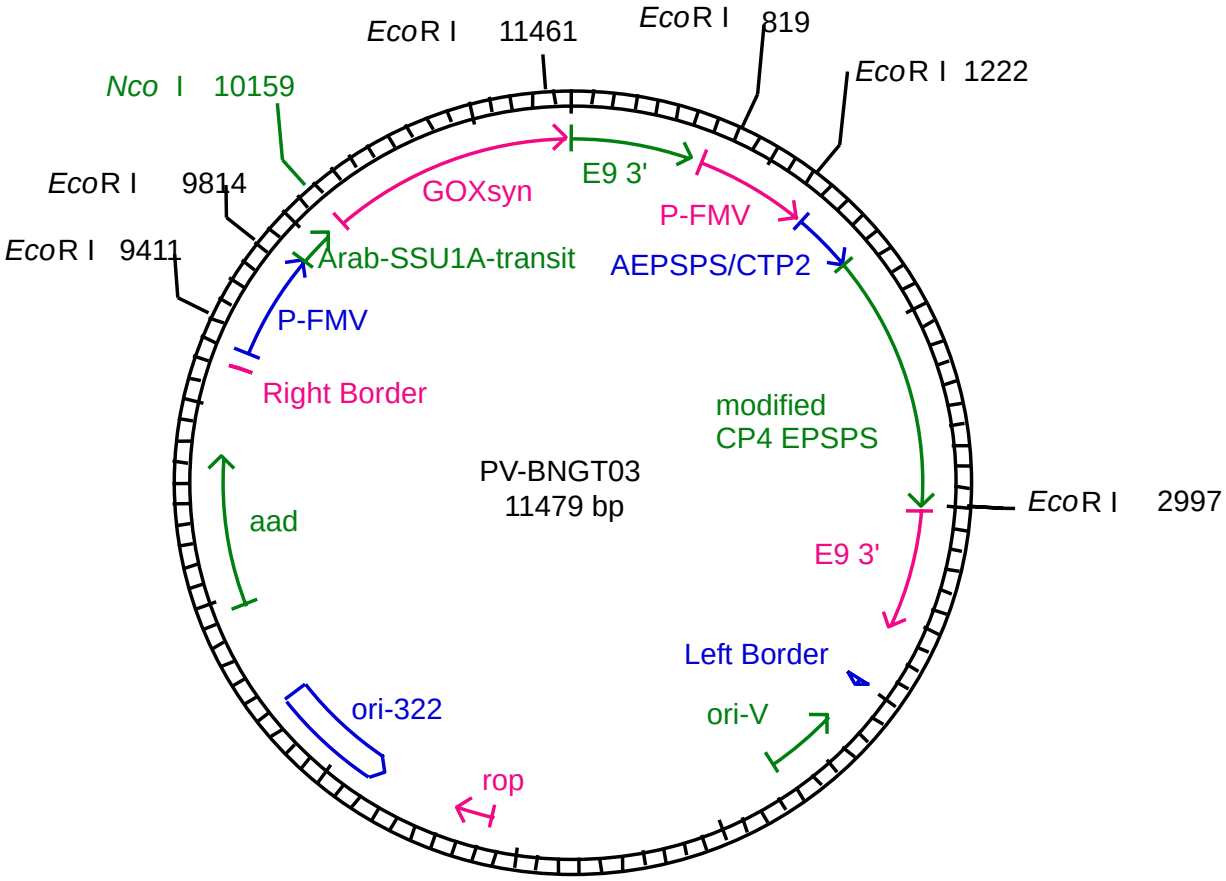

Figure 1 Map of plasmid PV-BNGT03 [3]

[3] All the rights pertinent to the information in the diagram above and the responsibility for the content rest upon Monsanto Japan Limited.

iii) Processes of rearing of living modified organisms

T-DNA region of plasmid vector PV-BNGT03 was introduced into the explants (leaves or flower buds) of Westar by the *Agrobacterium* method, and then regenerated individuals were obtained by culturing them in media containing glyphosate. In order to eliminate *Agrobacterium* from the regenerated plant, the regenerated plant was cultivated in media containing carbenicillin and paromomycin.

Regarding the obtained regenerated individuals, further selection was carried out based on the analysis of inserted genes and the expression level of the modified CP4 EPSPS protein. Tests in climate chamber and greenhouse were then carried out, and glyphosate tolerance and agronomic characters were examined in field tests. This recombinant oilseed rape was selected upon the comprehensive evaluation of these results (Figure 2).

The following shows the approvals received from organizations in Japan.

September, 2001: The safety of use of the cultivar for feed was approved in accordance with "Guideline for the safety evaluation of feed derived from recombinant-DNA plants, 6-(2)" by the Ministry of Agriculture, Forestry and Fisheries.

September, 2001: The safety of use of the cultivar for food, in accordance with "Safety Evaluation Criteria for Food and Additives derived from Recombinant-DNA Techniques" was ensured by the Ministry of Health, Labour and Welfare.

March, 2003: The safety of the use of the cultivar as feed, following "Procedure to Check the Safety of Feed and Additives Produced by Recombinant-DNA Techniques" was ensured by the Ministry of Agriculture, Forestry and Fisheries.

April, 2003: Based on the "Guideline for the use of recombinant in agriculture, forestry and fisheries", the compatibility to the guideline regarding recombinant being imported to Japan (used for processing and feed) was certified by the Ministry of Agriculture, Forestry and Fisheries.

[Not made available or disclosed to unauthorized person]

Figure 2 The process of rearing the oilseed rape RT200 tolerant to glyphosate herbicide

(4) State of existence of nucleic acid transferred in cells and stability of expression of traits caused by the nucleic acid

i) Location of copies of the introduced nucleic acids

On the chromosome

ii) Number of copies of the introduced nucleic acids and inter-generational inheritance stability of copies of the introduced nucleic acids

The inserted genes were analyzed by Southern blotting analysis and PCR analysis using the R3 and R4 generations. As a result, it was confirmed that one copy of T-DNA region consisting of the modified *cp4 epsps* gene expression cassette and *gox* gene expression cassette was inserted at one site in the genome DNA of this recombinant oilseed rape, and no other components were inserted in the genome DNA of this recombinant oilseed rape (Figure 3). The details on the analysis of inserted genes for this recombinant oilseed rape are shown in Annex 3.

iii) Nearby or separate location of multiple copies, if present, on the chromosome

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iv) Inter-individual or inter-generational expression stability under natural conditions with respect to the characteristics referred to specifically in (6) - (i)

As a result of analysis using multiple generations (R3 and R4), it was confirmed that the inserted genes were stably inherited to posterity (Annex 5). The hereditary stability of the inserted gene among this recombinant oilseed rape was confirmed by the tolerance test to glyphosate herbicide in each generation.

v) Existence of transmission routes and its scale, if it is possible that nucleic acids introduced via viral infection or other routes might be transmitted to wild animals and plants

It is only in gram-negative bacteria, such as *E. coli* and *A. tumefaciens*, that the plasmid PV-BNGT03 can sustain autonomous replication. Therefore, there is no possibility that the introduced nucleic acids might be transmitted to wild animals and plants that are not sexually compatible under natural conditions.

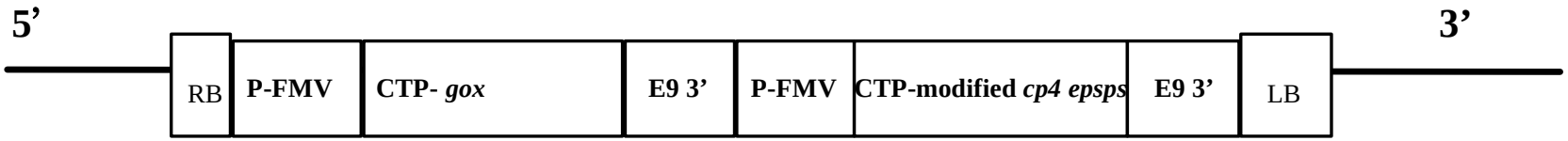

Figure 3 Genetic map of inserted genes in the oilseed rape RT200 tolerant to glyphosate herbicide [4]

[4] All the rights pertinent to the information in the diagram above and the responsibility for the content rest upon Monsanto Japan Limited.

(5) Methods of detection and identification of living modified organisms and their sensitivity and reliability

For the detection and identification of this recombinant oilseed rape, a qualitative PCR method has been developed where the DNA sequences of the inserted genes and the nearby regions of the plant genome are used as primers. This method makes it possible to specifically detect this recombinant oilseed rape (Annex 2).

(6) Difference from the recipient organism or the taxonomic species to which the recipient organism belongs

i) As a result of ELISA method, it was confirmed that the modified CP4 EPSPS protein, which is encoded by the modified *cp4 epsps* gene, and the GOX protein, which is encoded by the *gox* gene, are expressed in this recombinant oilseed rape (Annex 5).

ii) [5]Difference of this recombinant oilseed rape from the recipient organism of the non-recombinant control oilseed rape was examined at Zone No.1 of the isolated field in the National Institute of Vegetable and Tea Science in the period from May 2002 to March 2003. Regarding the assessment of environmental safety of this recombinant oilseed rape, cultivation test was attempted in the isolated field at Kawachi Research Farm, Monsanto Japan Limited, using R4 generation in response to the approval for conformity with the guideline obtained July 23, 2001 (13 Farmers No.669) (Annex 4). As a result, however, this recombinant oilseed rape exhibited significantly low germination rate and growth at the early stage. For this reason, the test scheduled in that year was abandoned and then the seed growing was conducted, preparing for a new test in FY 2002. Then the seeds collected from this recombinant oilseed rape (R5 generation) and the non-recombinant control oilseed rape were subjected to the isolated field tests in FY 2002.

(a) Morphological and growth characteristics

As can be found in Annex 5, differences in the following 18 items of morphological and growth characteristics were examined between this recombinant oilseed rape and the non-recombinant control oilseed rape, 3 repeats for each item and 5 individual plants for each repeat (7 individuals for each repeat regarding the items, plant height; time of flower completion; and plant shape or plant type): the uniformity of germination; germination rate; flowering period; plant height; time of flower completion; plant shape or plant type; the number of primary branches; the number of open flowers; harvest time; the number of pods formed; rate of open pods; length of a pod; width of a pod; the number of seeds per pod; color of grain; uniform excellence of grains (uniformity of grain size); shape of hilum; and weight of plant). Regarding the examination on the pods (length of a pod; width of a pod; the number of seeds per pod; color of grain; uniform excellence of grains; and shape of hilum), artificial pollination was conducted for a total of 10 flowers for each of 5 individuals per repeat, and 5 pods, which were found relatively better formed,

[5] All the rights pertinent to the information in the paragraphs (a) through (g) following this section and the responsibility for the contents rest upon Monsanto Japan Limited.

were selected for each for the examination. The artificial self-pollination was conducted by putting an adequate amount of pollens onto the stigma of the flowers selected at almost same positions between this recombinant oilseed rape and the non-recombinant control oilseed rape. The artificially self-pollinated flowers were marked with tags and other proper means, but left exposed from any covering in order to prevent possible decaying or falling pods due to the increased humidity.

As a result, regarding flowering period, the number of open flowers on the 7th day and 100-kernel weight, a statistically significant difference was observed between this recombinant oilseed rape and the non-recombinant control oilseed rape, though no difference was observed between them in the other items examined (Annex 5). The flowering period, in which a difference was observed between the recombinant oilseed rape and the non-recombinant control oilseed rape, was found 41.2 days on average for this recombinant oilseed rape compared to 40.0 days for the non-recombinant control oilseed rape. In addition, the number of open flowers on the 7th day was 108.7 for this recombinant oilseed rape and 95.2 for the non-recombinant control oilseed rape. Regarding 100-kernel weight, this recombinant oilseed rape exhibited about 409 mg on average and the non-recombinant control oilseed rape exhibited about 426 mg.

(b) Cold-tolerance and heat-tolerance at the early stage of growth

Regarding the cold-tolerance and heat-tolerance at the early stage of growth, comparison was made between this recombinant oilseed rape and the non-recombinant control oilseed rape. In the examination of heat-tolerance, the seedlings collected at the 1st leaf stage of this recombinant oilseed rape and the non-recombinant control oilseed rape (5 plants for each×3 repeats) were put in a climate chamber at 35℃ (3,800 lux, 14-hours day length) for 25 days. Then, leaf age, plant height, and the above-ground fresh weight and dry weight of the plant cut off at the ground level were examined. As a result, no statistically significant difference was observed between this recombinant oilseed rape and the non-recombinant control oilseed rape in the leaf age, plant height, fresh weight and dry weight (Annex 5).

For the cold-tolerance, the seedlings collected at the 1st leaf stage of this recombinant oilseed rape and the non-recombinant control oilseed rape (5 plants for each×3 repeats) were put in a climate chamber at 5℃ (3,000 lux, 12-hours day length) for 45 days. Then, leaf age, plant height, and the above-ground fresh weight and dry weight of the plant cut off at the ground level were examined. As a result, no statistically significant difference was observed between this recombinant oilseed rape and the non-recombinant control oilseed rape in leaf age, plant height, fresh weight and dry weight (Annex 5).

(c) Wintering ability and summer survival of the matured plant

In the isolated field tests, it was observed that dying started after ripening in autumn for this recombinant oilseed rape sown in spring similarly as the

non-recombinant control oilseed rape. Based on the above, an overwintering test for the matured plant of this recombinant oilseed rape was not carried out.

(d) Fertility and size of the pollen

To examine the fertility (maturity) and size of the pollens, pollens were collected from the 3-repeats zones selected for examination of growth characteristics of this recombinant oilseed rape and the non-recombinant control oilseed rape during the flowering period (3 flowers for each per repeat), and stained with acetocarmine. As a result, fertility of the pollen of this recombinant oilseed rape and the non-recombinant control oilseed rape was found 97.8% and 98.0% respectively, showing no statistically significant difference between them. In addition, no difference was also observed between this recombinant oilseed rape and the non-recombinant control oilseed rape in size and shape of the pollen (Annex 5).

(e) Production, shedding habit, dormancy and germination rate of the seed

Regarding seed production, the differences between this recombinant oilseed rape and the non-recombinant control oilseed rape have been examined for the number of pods formed and the number of seeds per pod as mentioned in (a) Morphological and growth characteristics. As a result, in all items examined, no statistically significant difference was observed between this recombinant oilseed rape and the non-recombinant control oilseed rape (Annex 5).

Regarding shedding habit, the differences between this recombinant oilseed rape and the non-recombinant control oilseed rape have been examined for the rate of opened pods as mentioned in (a) Morphological and growth characteristics. As a result, regarding the rate of opened pods, no statistically significant difference was observed between this recombinant oilseed rape and the non-recombinant control oilseed rape (Annex 5).

To evaluate dormancy and germination rate, the seeds harvested for individual repeats after artificial self-pollination of this recombinant oilseed rape and the non-recombinant control oilseed rape were sown in a petri dish (30 grains each per repeat$\times$3 repeats), which was put stationary in an incubator at 25°C to examine the germination rate. As a result, the harvested seeds of this recombinant oilseed rape and the non-recombinant control oilseed rape exhibited the germination rate of 93.0% and 94.1% respectively, indicating no statistically significant difference (Annex 5).

(f) Crossability

Three (3) plants of the non-recombinant control oilseed rape adjacent to the zone of this recombinant oilseed rape were selected from each of four (4) sites (Annex 5), and all available seeds from each plant were collected at harvest time and sent to the Kawachi Research Farm, Monsanto Japan Limited. In a containment greenhouse at the Kawachi Research Farm, Monsanto Japan Limited, the seeds were sown by individual plant, and the seedlings (at 2nd to 3rd leaf stage) raised from the seeds were sprayed with glyphosate herbicide

(dose of 250 mL/10a) to identify the number of the surviving individuals among the total number of seedlings. Then, regarding the surviving individuals as hybrids, natural crossability of this recombinant oilseed rape was evaluated.

As a result, the incidence of the glyphosate-tolerant plants in the zone adjacent to this recombinant oilseed rape (＝natural crossability) was found between 2.1 and 3.0%, and the natural crossability among the total number of seedlings of 2,976 was 2.7% (Annex 5). In the typical fields of the non-recombinant oilseed rape, the natural crossability is known between 10 and 20% (Reference 17). Therefore, it was considered that the crossability of this recombinant oilseed rape observed in this test would not substantially exceed the typical crossability of the non-recombinant oilseed rape.

In addition, based on the fact that insects (primarily honeybees) play a key role as the means for transferring pollens (Reference 6), examination was made to identify flower-visiting insects for individual repeats in 2 days, July 3 and July 19, during the flowering period of this recombinant oilseed rape and the non-recombinant control oilseed rape, two times on each day in the morning and afternoon. As a result, the flower-visiting insects trapped in the zones of this recombinant oilseed rape and the non-recombinant control oilseed rape included two types of honeybees, Japanese honeybees (*Apis cerana*) and Western honeybees (*Apis mellifera*), and no significant difference was observed in the number between the two types of honeybees trapped (Annex 5).

(g) Productivity of harmful substances

In order to check that this recombinant oilseed rape does not produce any substances which can affect other plants and/or microorganisms in soil, soil microflora test, succeeding crop test and plow-in test were carried out. As a result, for all items tested but the above-ground fresh weight in the succeeding crop test, no statistically significant difference was observed between this recombinant oilseed rape and the non-recombinant control oilseed rape (Annex 5).

In addition, as a result of evaluation on the coverage of flora generated in the cultivation zones of this recombinant oilseed rape and the non-recombinant control oilseed rape based on visual observation, no difference was observed between this recombinant oilseed rape and the non-recombinant control oilseed rape (Annex 5).

II. Review by persons with specialized knowledge and experience concerning Adverse Effect on Biological Diversity

A review was made by persons with specialized knowledge and experience concerning Adverse Effect on Biological Diversity (called Experts) for possible Adverse Effect on Biological Diversity caused by the use in accordance with the Type 1 Use Regulation for Living Modified Organism based on the "Law concerning the Conservation and Sustainable Use of Biological Diversity through Regulations on the Use of Living Modified Organisms." Results of the review are listed below.

1. Item-by-item assessment of Adverse Effect on Biological Diversity

(1) Competitiveness

Oilseed rape *(Brassica napus* L.) to which the recipient organism belongs was introduced to Japan in early Meiji period, and it is reportedly growing on river banks, along roadsides, in the surroundings of seed off-loading harbors, and in other such areas. It is generally known that oilseed rape could become self-seeding in the environments such as roadsides and abandoned plant sites maintained with regular care. However, it is also known that oil rape seed could not survive the competition with perennial wild plants and thus it is difficult to become self-seeding under natural conditions left almost uncontrolled. Therefore it is not regarded as an aggressive introduced species which could exterminate Japanese native species of plants and cause Adverse Effect on Biological Diversity.

This recombinant oilseed rape has the tolerance to glyphosate herbicide conferred by the transferred modified *cp4 epsps* and *gox*, but it is hard to consider that the glyphosate will function as a selection pressure in the natural environment. In addition, various traits relating to competitiveness were examined in the isolated fields in Japan. Regarding the flowering period, the number of open flowers on the 7^{th} day and 100-kernel weight among the items examined, a significant difference was observed between this recombinant oilseed rape and the non-recombinant oilseed rape. However, it is hard to consider that this difference can cause this recombinant oilseed rape to become competitive and have adverse effect on biological diversity.

Even if this recombinant oilseed rape, growing at roadsides or in other areas where herbicide glyphosate is used for the purpose of weed control, could survive the herbicide sprayed, such areas are in general exposed to non-selective weed control, and this recombinant oilseed rape could be removed by use of any weed killers other than the glyphosate and/or mowing. In addition, as mentioned above, it is not considered that the recombinant oilseed rape becomes more dominant by utilizing herbicide tolerance under the pressure of natural selection than the non-recombinant oilseed rape does. Consequently, it is considered to be extremely low that, even if this recombinant oilseed rape could survive, it propagates beyond the roadsides and becomes dominant in the natural conditions kept away from human intervention.

Based on the above understanding, wild species likely to be affected cannot be specified, and it was judged to be valid that the applicant concluded that there is no risk of Adverse Effect on Biological Diversity attributable to competitiveness.

(2) Productivity of harmful substances

Conventional oilseed rape is known to produce erucic acid and glucosinolate in the seeds as harmful substances to human and other mammals. Westar, the host of this recombinant oilseed rape, is one of the cultivars known as Canola which features reduced contents of erucic acid and glucosinolate achieved by selective breeding. In fact, the component analysis of this recombinant oilseed rape has confirmed that erucic acid and glucosinolate contents fall within the threshold specified for Canola cultivars.

This recombinant oilseed rape produces the modified CP4 EPSPS protein and GOX protein which contribute to confer the tolerance to glyphosate, though there is no report showing these proteins function as harmful substances.

EPSPS protein is an enzyme which catalyzes the shikimate pathway for aromatic amino acid biosynthesis, but it is known that EPSPS is not a rate-determining enzyme in this pathway, and as such it is not considered that enhanced EPSPS activity will increase the concentration of aromatic amino acids, the end products of this pathway. In addition, EPSPS protein is an enzyme which specifically reacts with the phosphoenolypyruvate (PEP) and shikimate-3-phosphate (S3P), therefore it is not considered that CP4 EPSPS protein catalyzes reaction of any other substances and produces different substances. GOX protein is an enzyme which catalyzes the reaction to degrade glyphosate into aminomethylphosphonic acid and glyoxylate, but it has high substrate specificity, and it is considered that its modified type GOX protein does not act on any other metabolic systems of plants.

In addition, in Japan, the productivity of harmful substances (including secretion from roots to affect the other plants, secretion from roots to affect microorganisms in soil, and substances in the plant body to affect the other plants after dying out) has been investigated in the isolated fields., However, it is not recognized that there is significant difference between this recombinant oilseed rape and the non-recombinant oilseed rape.

Based on the above understanding, wild species likely to be affected cannot be specified, and it was judged to be valid that the applicant concluded that there is no risk of Adverse Effect on Biological Diversity attributable to productivity of harmful substances.

(3) Crossability

In natural environment in Japan, a number of plants of the family *Brassicaceae* are growing, though known species that can be crossed with oilseed rape (*Brassica napus* L.) include *B. rapa* L. (turnip, Komatsuna, conventional rapeseed, etc.) of the genus *Brassica*; *B. juncea* (L.) Czern (mustard, leaf mustard, etc.); *B. nigra* (L.) W.D.J.Koch (black mustard) and *Raphanus raphanistrum* L. (radish) in addition to oilseed rape itself.

Oilseed rape, *B. juncea*, *B. nigra*, and *R. raphanistrum* are all introduced species reportedly brought into Japan artificially after Meiji period. In addition, *B. rapa* is also a cultivar-derived introduced species though it was introduced to Japan in olden times. As such, these are not specified as wild species likely to be affected.

Based on the above understanding, wild species likely to be affected cannot be specified, and it was judged to be valid that the applicant concluded that there is no risk of

Adverse Effect on Biological Diversity attributable to crossability.

(4) Additional information

i) Possibility of indirect Adverse Effect on Biological Diversity attributable to crossing

Regarding the possibility of indirect Adverse Effect on Biological Diversity caused by crossing with related species including this recombinant oilseed rape and oilseed rape in natural environment in Japan, the following (a) and (b) were evaluated.

(a) Crossbreds produced by crossing become competitive and destroy population of other wild animals and plants.

(b) Transferred genes from crossing act to reduce population of related species, thereby compromising the preservation of population of insects and other wild animals and plants which rely on the related species.

1) Possibility of indirect Adverse Effect on Biological Diversity caused by crossing with oilseed rape (*B. napus*)

Cross pollination of oilseed rape by wind- or insect-pollination is reported 5 to 30%. As a result of crossability tests in isolated fields in Japan and tests in non-closed system greenhouse for fertility of pollen, dispersion of pollen, flower-visiting behavior of honey bees and other such reproduction characteristics, it is suggested that this recombinant oilseed rape is comparable to the non-recombinant oilseed rape in the crossability.

Based on above understanding, there is a possibility that this recombinant oilseed rape could grow at roadsides or other places from fallen seeds or pollens dispersed from cultivated areas and cross with oilseed rape growing at roadsides or riverbanks, thereby causing crossbreds.

However, as discussed in i), it is unlikely that the glyphosate herbicide tolerance conferred by the modified *cp4 epsps* and *gox* transferred into this recombinant oilseed rape offers competitiveness under natural environment, therefore, it is considered extremely low that the crossbreds between this recombinant oilseed rape and the non-recombinant oilseed rape become more competitive compared to the non-recombinant oilseed rape and exterminate population of other wild animal and plant species.

In addition, it is hard to consider that introgression of herbicide tolerant genes rapidly reduces the population of oilseed rape.

2) Possibility of indirect Adverse Effect on Biological Diversity caused by crossing with *B. rapa, B. juncea, B. nigra* and *R. raphanistrum*

B. rapa, B. juncea, B. nigra, and *R. raphanistrum* are all growing voluntarily in wastelands and roadsides in Japan. Overseas literature search indicates that crossability with oilseed rape is 0.4 to 13% for *B. rapa*, 3% for *B. juncea*, and very low for *B. nigra* and *R. raphanistrum*. In addition, even in the event of crossing, these related species are different from each other in the number of chromosomes and compositions, and as such it is suggested that there is a mechanism of crossbred breakdown causing significant decreased fertility of pollens or seeds of inter-specific crossbreds produced by crossing.

In the isolated field tests in Japan, crossability of this recombinant oilseed rape with *B. napus* has been examined with the result that this recombinant oilseed rape is comparable to the non-recombinant oilseed rape in the crossability. Therefore, it is considered that the crossability character of this recombinant oilseed rape with related species including *B. rapa, B. juncea, B. nigra* and *R. raphanistrum*, for which no test has been conducted, is not significantly different from that of the non-recombinant oilseed rape.

Consequently, it is unlikely that this recombinant oilseed rape could cross with *B. rapa, B. juncea, B. nigra*, and *R. raphanistrum* and even in the event of crossing, it is considered extremely low that the transferred genes could introgress into the population of these species.

Based on the above understanding, it was judged to be valid that the applicant concluded that there is no risk of indirect Adverse Effect on Biological Diversity attributable to crossability.

ii) Possibility of Adverse Effect on Biological Diversity attributable to occurrence of recombinant virus

For this recombinant oilseed rape, 35S promoter of *Figwort mosaic virus* (FMV) of the genus *Caulimovirus* is used. In Japan, FMV is not distributed, but as the virus belonging to the genus *Caulimovirus*, *Carnation etched ring virus* (CERV), *Cauliflower mosaic virus* (CaMV), *Dahlia mosaic virus* (DMV), and *Strawberry vein banding virus* (SVBV) are distributed, and among these, CaMV is known to make the genus *Brassica* the recipient organism. In response to this fact, a possibility was examined whether recombinant virus could emerge through homologous recombination in which 35S promoter of FMV is caught into CaMV.

However, the homology of nucleotide sequences between FMV35S promoter used in this recombinant oilseed rape and CaMV35S promoter is 10% or less for the 553bp nucleotide sequence equivalent almost to the total length, though there exists 56bp region showing the homology of 68%. Therefore, it is considered extremely low that homologous recombination could occur. Consequently, it is also considered extremely low that recombinant virus will emerge.

Based on the above discussion, it was judged to be valid that the applicant concluded that there is no risk of Adverse Effect on Biological Diversity attributable to occurrence of recombinant virus.

2. Conclusion

Based on the above understanding, the Biological Diversity Risk Assessment Report concluded that there is no risk that the use of this recombinant oilseed rape in accordance with Type I Use Regulation causes Adverse Effect on Biological Diversity. The conclusion above made by the applicant was judged to be valid.

【Reference】

[Not made available or disclosed to unauthorized person]

Annex List

Annex 1 Factual investigation on falling oilseed rape imported for active ingredient

(Reference address) http://www.s.affrc.go.jp/docs/press/2004/0629.htm

Annex 2 Sequence of the Genetic Elements in PV-BNGT03
[Not made available or disclosed to unauthorized person]

Annex 3 Molecular Analysis of Roundup Ready Canola Event RT200

[Not made available or disclosed to unauthorized person]

Annex 4 Program for application of recombinant plant: Recombinant rapeseed RT200 line tolerant to glyphosate herbicide (Application in a simulated environment)

[Not made available or disclosed to unauthorized person]

Annex 5 Program for application of recombinant plant: Recombinant rapeseed RT200 line tolerant to glyphosate herbicide (Application in an open system)

[Not made available or disclosed to unauthorized person]